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THE ULTRASTRUCTURE OF BACTERIA

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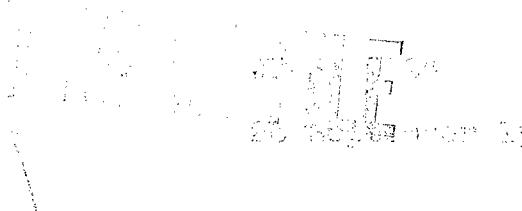
By A. P. Pekhov

- USSR -

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THE ULTRASTRUCTURE OF BACTERIA

Following is the translation of an article by A. P. Pekhov entitled "Ul'trastruktura Bakteriy" (English version above) in Vestnik Akademii Meditsinskikh Nauk SSSR (Herald of the Acad. Med. Sci. USSR), Vol. XV, No. 5, Moscow 1960, pages 43-54.

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Electron microscopy as a method of structural analysis has provided an immense amount of data on the submicroscopic structure of bacteria.

It is difficult at present to find a laboratory where electron microscopy is not employed in the study of the bacterial characteristics under various environmental conditions. The sphere of the utilization of this method for the study of bacterial ultrastructure is constantly widening. In the Department of Immunobiology (Department Head: Active Member of the Acad. Med. Sci. USSR, Prof. N. N. Zhukov-Verezhnikov) of the Institute of Experimental Biology, Acad. Med. Sci. USSR, electron microscopy has been employed for a number of years. During that time we have carried out work on the study of the submicroscopic cellular structure of a number of varieties of bacteria, especially intestinal bacteria. Work has been carried out on an ultrastructural study of intact as well as disintegrated cells, in connection with the effect of physical, chemical and biological factors on them. The present article summarizes some of the results which have obtained, elucidates the data of other researchers, and offers a review of information which determines the present level of knowledge of the submicroscopic structure of bacteria.

Before analyzing the factual data, it is expedient to elucidate the possibilities and limitations of electron microscopy as well as the problems connected with preservation of the minute structures of bacteria during their preparation for ultramicroscopic study.

Modern technical means enable us to study the submicroscopic bacterial structure not only by observing intact

cells and cells destroyed by various physical, chemical and biological factors, but also by preparing separate ultramicroscopic sections. The preparation of ultrafine sections, which is tantamount to a dissection of the bacteria, reveals the minute composition of the inner cellular structures.

The aforementioned possibilities determine also the methods of approach to the study of the submicroscopic structure of microorganisms. These methods are basically as follows.

1. Study of intact bacterial cells, prepared simply by placing a suspension of them in a spoon, or growing them on a colloidal film until a second cellular layer in the microcolony appears. In this case it is possible to obtain only general information on the form and size of bacteria and on the localization of cellular components, for example, the flagella. The inner structure remains hidden and inaccessible to visual analysis. During the early studies of bacterial ultrastructure this method was widespread. At present, however, it is useful primarily for characterizing those microorganisms the electron-microscopic images of which are still unknown.

2. Destruction of bacteria by means of physical, chemical and biological actions (mechanical grinding, sound vibrations, salts of heavy metals, aging, antibiotics, phages, etc.), followed by a structural analysis of the products of cell disintegration with subsequent reconstruction of the general picture of arrangement of its separate components.

This approach is used when it is desired to single out the essential components in order to analyze their minute characteristics, i. e., to carry out, practically, an analysis of the inner content of the bacteria, even though the integrity of the natural arrangement of structures is disturbed in the process.

3. Dissection of bacterial cells by the method of ultrafine sections and the study of the inner cellular content thus exposed. The complete method of ultrafine sections has been developed only relatively recently, but its use has had a substantial effect on the development of electron microscopy. The ultrafine sections, averaging 100 Å in thickness, opened up the possibility of realizing the high resolving power of these microscopes, and thus ensuring an accurate study of the geometry and molecular structure of the components exposed. This is extremely important in the study of such cellular components as, for example, membranes which consist of several layers or aggregates of macromolecules and thus represent in essence supramolecular entities.

In high-power electron-microscope examinations it is necessary first of all to take in consideration the fixation

of the preparation, which is the essential condition for the preservation of the ultrastructures. In the preparation of histological slides for luminous microscopy, the significance of fixation cannot be fully determined, since structural changes, which occur during fixation, are not elicited by means of an illumination microscope, which has at best a resolving power of about 2,000 Å. In electron microscopy the results of improper fixation are detected instantly.

Osmium tetroxide proved to be of value in fixing objects and, in correctly chosen concentrations, it brings out pictures which are not artifacts. This has been proved by the investigations of many authors engaged in the biological study of ultrastructures. It turned out also that the fixation of tissues can be achieved best at low temperatures on the order of one to five degrees. However, this is not critical in the case of bacteria.

In order to avoid artifacts in bacterial studies, it is important to effect the fixation of cells at the moment corresponding to the period of their development being investigated. The available data indicate that the material fixed under such circumstances retains the properties which correspond to the structural conditions in live cells.

In the dehydration and fixation of objects, the procedures themselves, as well as the materials, apparently are not critical. The fixation materials are of importance only in that the preparation, placed in the column of the microscope, is subjected to the heating effect of the electronic beam. But here, also, practical experience has shown that with methacrylates and araldite, which are used now as fixation materials, the thermal conditions created by the electronic beam concentration are such that no discernible changes are induced in the enclosed object.

Thus, we may consider that the problem of preserving the structural biological material for electron-microscopic examination is basically solved satisfactorily at the present time.

The ultrafine sections make it possible to replace purely descriptive characterizations of objects with thorough analyses of submicroscopic and molecular structure. Through the use of ultrafine bacterial sections, objective information can be obtained on ultrastructure within limits inaccessible to other methods. Such information includes (a) data relating to the geometry of elementary structural components of intact bacteria, which, consisting of several molecules, are joined into supramolecular structures -- these structures can be measured, provided their sizes are between 20 and 15 Å, and the supporting membranes and their contrasting properties contribute to the identification of these

structures; (b) data on the inner organization of such elementary structures, as well as on the particles which consist of aggregates of still smaller units; (c) data for three-dimensional visualization of the structural organization of supermolecular components, based on the analysis of topographic correlations in their natural arrangement in the cell.

Such qualitative and quantitative information supplied by the visual inspection of ultrafine sections may be supplemented also by an actual count of the elementary components in a unit of volume of the bacterial cell cytoplasm.

Thus, the analysis of bacterial ultrastructure is based at present on methods which yield not only qualitative but quantitative characteristics of the fine structure of the formations disclosed. The achievement of preserving the structure of the biological material during preparation of sections, and also the reproducibility of the images, speak for the authenticity of these images.

If we analyze the bacterial cell from the point of view of its components, we can observe that its organization does not differ, on the whole, from the cellular organization of higher forms. The constituents of the bacterial cell include the membranes, organs of locomotion in the mobile forms (flagella), cytoplasm, nuclear structure, and the cytoplasmic enclosures.

Membranes. Not very long ago there were various opinions as to the true character of the bacterial membrane, since the light-optical observations of colored preparations could not elicit all the details of their structure. Before electron-microscopic studies were carried out, it seemed difficult to visualize the true connection between the membrane and the morphological bacterial entity, as well as the dependence of the bacterial form on the character of the membranes.

Although the study of intact cells by means of electron microscopy enables us to obtain images of bacterial membranes, this method of observation cannot provide more detailed information on their structure, since the membranes appear on the images of the intact cells in the form of flat, undifferentiated formations. The most complete information on the character and structure of the bacterial membrane can be obtained only by examining ultrafine bacterial sections.

In order to study the membranes, we prepared sections of cells of *Bacterium coli* B. Fig. 1 shows pictures of sections of separate bacteria of this strain. On studying these pictures it becomes evident that the membrane has a dual character. Its external part, or the membrane proper (plus the cellular wall), is about 60 Å thick -- a thickness which,

as is known, corresponds to the diameter of a small, spherical protein molecule. Here it is necessary to point out certain discrepancies between this figure and the figure obtained as a result of study of the membrane of the same microorganism by Birch-Andersen, Maale, and Sjöstrand (1953). According to their data, the cell wall of *B. coli* B is 100 Å thick. Other authors also obtained somewhat larger figures when they attempted to measure the thickness of the membrane in various microorganisms of the intestinal group. Consequently, we can assume that the thickness of the membrane (cell wall) varies between 60 and 100 Å.

The inner part of the membrane is a formation that tightly envelopes the cytoplasm. Since this part of the membrane is very thin, it can be regarded as a cytoplasmic membrane in the form of a layer of spherical macromolecules.

Between the cell wall and the cytoplasmic membrane there is a space about 120 Å thick, which corresponds approximately to the thickness of a triple layer of lipid molecules. Thus the membrane consists of an external layer, the bacterial wall, and an inner one, the cytoplasmic membrane.

Data are available on the relation between the mass of the cell wall and the mass of the bacterium. The cell wall constitutes a considerable part of the total bacterial mass: according to calculations by Cooper, Rowley, and Dawson (1949), made on *staphylococcus* cells, the cell wall takes up about 20 percent of the dry weight of the bacterium.

According to the data of Salton (1956), this ratio is much lower in gram-negative microorganisms.

Many studies, our experiments included, have been directed toward the elucidation of the physical properties of cell membrane components for the purpose of clarifying the roles of both the cell wall and the cytoplasmic membrane, as well as the relation between them.

The rigid character of the cell wall was pointed out by the experiments of Salton and Williams (1954). The authors made preparation of isolated cellular membranes by the method of freeze-drying (which, as is known, preserves the structures to a considerable extent and eliminates possible artifacts), and found that the membranes of *bacillus*-like microorganisms have a typical cylindrical shape.

In Fig. 2 are shown pictures of empty cellular membranes obtained by us in experiments on *B. coli* lysis. Deprived of the cytoplasm and its enclosures, the empty membranes appear in the photograph in the form of sacs in the shape of bacterial cells (cylindrical). It is important to stress the fact that these preparations were made by simple air-drying. These experiments show that the cellular wall can be regarded as a fairly rough shell enveloping the cell and giving it a corresponding form -- bacillary, for example. When the inner

content of the cell is removed, the shell does not change its form, but if the bacteria grow in size, as happens, owing to various factors (antibiotics, phage), the membrane expands. The fact that the membrane truly represents a rigid shell was demonstrated conclusively in experiments in which bacterial protoplasts were obtained. During the process of obtaining protoplasts, according to Weibull (1953), while the destruction of the cellular wall takes place in a saline medium, the bacteria lose their bacillary form and assume a spherical form in which the cytoplasmic membrane is retained. The transformation takes place owing to the fact that, during the destruction of the wall, the cytoplasmic content is liberated together with the cytoplasmic membrane, and the latter, because of its low rigidity, permits the cytoplasm assume a spherical form as the result of the difference in the pressures inside the cell and in the external medium. The function of the cytoplasmic membrane is to protect the cytoplasm. When the integrity of the cytoplasmic membrane is impaired, the disintegration of the protoplasm takes place even in a saline medium.

Analysis of the data on the structure of the membrane in Gram-positive bacteria shows that it does not differ from that of Gram-negative bacteria. Their chemical compositions are, however, different. The Gram-positive bacteria, in contrast to the Gram-negative ones, are characterized by the absence in the membrane of aromatic and the sulfur-containing amino acids proline, histidine and arginine. The membranes of Gram-negative bacteria contain more lipids (Salton, 1958), which may account for the width of space between the wall and the membrane.

Flagella. An interesting characteristic of the flagellate apparatus is the fact that its removal does not result in the destruction of the cell. The latter remains viable and continues to grow and develop. The thickness of the flagella varies, but only slightly, depending on the bacterial species. According to our observations, the flagellar thickness of *B. coli*, in preparations shaded against metal, equals 200 Å. According to measurement by other authors, the flagellar thickness in the case of *Protæus vulgaris* equals 120 Å.

The flagellar length also varies, equalling on the average several microns. The submicroscopic structure of flagella proves to be very complex. Externally, they show a peculiar striated structure which reflects their spiral form. Leifson, Carhart, and Fulton (1955) demonstrated that the length of the wave -- i. e., the distance between the apices of two turns of a spiral -- equals 80 Å. This "wavings" in

in the opinion of those authors, is the result of not only genetic factors but of environmental factors as well. It is sufficient to change the pH from 7.2 to 6.2 to effect an immediately noticeable contraction of the length of the "wave."

Electron microscopy fully confirmed the previously held idea that the flagella originate in the protoplasm and find their way out through the cellular wall. At the point of origin of the flagella in the protoplasm there are so-called basal granules 400 Å in diameter.

In studying the flagellar apparatus we found that, in addition to the usual flagella, the cells of *B. coli* aerogenes contain peculiar, small flagella resembling cilia. These cilia are very thin and short, and do not curl. It is of interest that this type of flagella is repeated genetically in regenerated secondary cultures from the filterable forms of cells of the original strain of this microorganism: the secondary culture cells as well as the cells of the original strain have, in addition to the usual flagella, cilia situated over a considerable area of the surface (Fig. 3). The role of these flagella is obscure, though their presence is characteristic of more than one bacterial species, since they have been found in a number of other microorganisms also (Smith, 1954; Howrink and Iterson, 1950; Brinton, Bouzell, and Lauffer, 1954).

Cytoplasm. Bacterial cytoplasm has a fine-grained character. That it is composed of fine granules is now perfectly obvious and has been corroborated by the results of direct as well as indirect examination. Of course, the method of staining the bacteria and the use of a light microscope proved to be unfit for the solution of this problem. Despite the fact that the cytoplasm has been studied for a very long time, the establishment of its fine-grained character became possible only recently with the introduction of the electron-microscopic study of bacteria, when direct observation of the granular substance became possible with bacterial sections.

Careful fixation of bacteria preserves the fine-grained character of the cytoplasm (see Fig. 1); the contours of isolated granules are clearly visible. In this case they are 100 Å in diameter. According to the data of Bradfield (1956), who had studied the submicroscopic structure of the cytoplasm of several species of microorganisms, the diameter of the granules is 100 to 200 Å. It is necessary to mention that the granules are visible in all published photographs of ultrafine sections of bacteria which were taken at a high power of the microscope and after satisfactory fixation of the material.

One of the weightiest proofs in favor of the fact that these granules truly represent the cytoplasmic base are

the results of the study of the products of bacterial disintegration.

In 1956, as a result of treating a suspension of *Bacterium suispestifer* cells with sound vibrations on the order of 10 to 20 kilohertz, we obtained substances which consisted basically of fine-grained elements, which had formed as a result of disintegration of the protoplasm. The size of each of these granules was 100 to 450 Å. There were also encountered unusual protoplasmic "flaps" consisting of several granules which had not yet been dispersed (Fig. 4). The force of cohesion between these granules is apparently small, since the cytoplasm is less resistant to sound vibrations and it disintegrates more rapidly than do the flagella or membranes, for example. The destruction of bacteria by a phage (1957) is analogous: the cytoplasmic areas, liberated from newly formed phagoparticles, are transformed into fine-grained elements capable of passing through bacterial filters. Schachman, Pardee and Stanier (1952) studied the homogenates of several bacterial species by the method of ultracentrifugation, and demonstrated that the species consist of three macromolecular components with sedimentation constants of approximately 40, 29 and 5 units, according to Swedberg.

Further studies demonstrated the presence of oxidizing enzymic systems in the granules (Repaske, 1954; Billen and Volkin, 1954). The granules are elicited on bacterial sections which are fixated not with osmium but with formalin; consequently, it cannot be contended that the granules are artifacts caused by the condensation of osmium particles in the cytoplasm.

The data available on the structure of cytoplasm enable us to speak with certainty of the granular character of the cytoplasm. Only the character of the bond between separate granules remains obscure, and data are lacking on the fine structure of each separate granule.

Cytoplasmic enclosures. As the basic cytoplasmic enclosures we must consider the nuclear structures and the large cytoplasmic granules that are called in the literature "polar caps," or elements equivalent to mitochondria. The study of these cytoplasmic enclosures is of very considerable interest at present. While the problem related to nuclear structures has been a subject of study for a long time, the problem of bacterial mitochondria was posed only recently. It is necessary to state that the interest in these problems is due to a number of conclusions based on available experimental data. These data are of extreme importance in many sections of microbiology, primarily, in cytogenetics and the physiology of

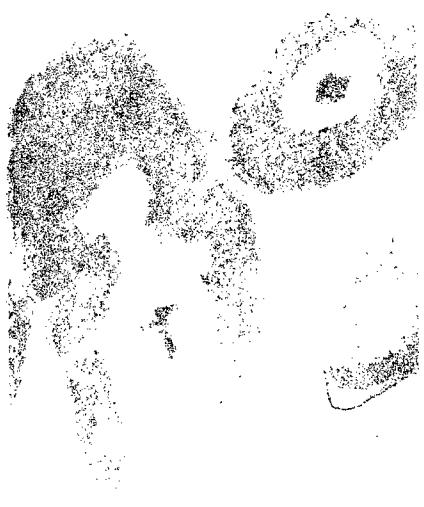
(a) Nuclear structures. The history of the problem and basic data on bacterial nuclei are most comprehensively presented in the excellent work of M. A. Peshkov (1955). The experimental material on nuclear structures of bacteria is very rich and many-sided; nevertheless, certain researchers still think that the presence of nuclear structures in bacteria has not yet been proved. As the first and basic premise in favor of their point of view they contend that the structures assumed to be nuclei are artifacts, produced in the process of staining the bacteria for nuclei, or that the so-called "polar caps" are taken for nuclei.

We must state that, in the study of stained bacterial preparations, none of the systematic improvements have led, in essence, to the final solution of the question of the existence of nuclei; answers to many problems are still obscure. A number of difficulties are associated with the analysis of intact cells by electron microscopy also. In observing bacteria by the method of electron microscopy, many researchers have reported that certain bright areas encountered on the cellular surface correspond to nuclear structures. Despite the fact that in many instances the specific positive staining of nuclear substances was reported, the question has remained to a certain degree unanswered. The main difficulty lies in the fact that the superficial cellular layers are impenetrable to electrons, so that the inner structures cannot be revealed, and thus the bright areas, though less solid, can be treated as nuclei only conjecturally. It is methodologically impossible to study the same bacterial cell under both electron and light microscopes; therefore the bright spots can be explained as having some other origin.

The most practical and reliable study of nuclear structures is effected on ultrafine sections. In carrying out this work, we assumed that there is not need for any supplementary treatment of bacteria because the inner structures are revealed via removal of the upper layers of the bacterial cell by the method of ultrafine sections. If there really are nuclei present, they ought to reveal themselves in the form of unique discreet formations on the surface of the sections.

We studied the nuclear structure in ultrafine sections of *B. coli* aerogenes and *B. coli* B cells. The electron-microscopic observations of sections of *B. coli* aerogenes cells were supplemented by an optical light study of the cells stained by the Romanovskiy-Gimza method.

Preliminary study of whole cells stained according to the Romanovskiy-Gimza and Fel'gen methods showed that in the bacterial protoplasm there really were formations which, in



0.5 μ

Fig. 1. Ultrafine sections of cells.



Fig. 2. An empty membrane of a dividing cell and *B. coli*.

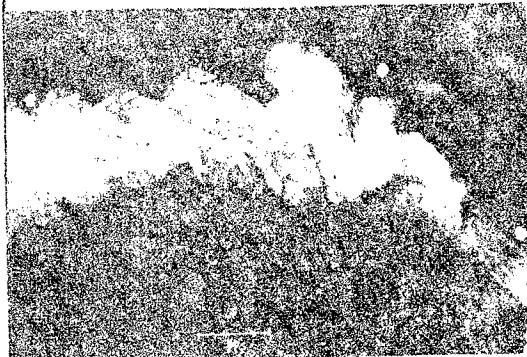


Fig. 3. Cells of a secondary culture of *B. coli* with two types of flagella.

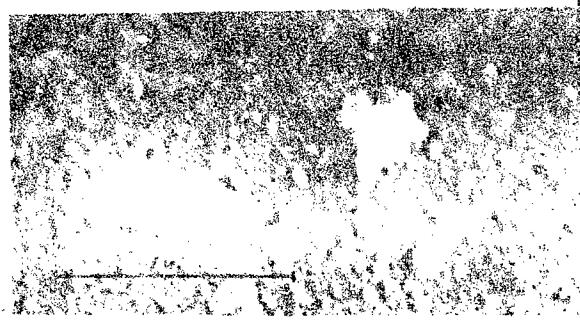


Fig. 4. Small cytoplasmic granules and "flaps" of *Bacterium suipestifer* cytoplasm after destruction by sound waves.

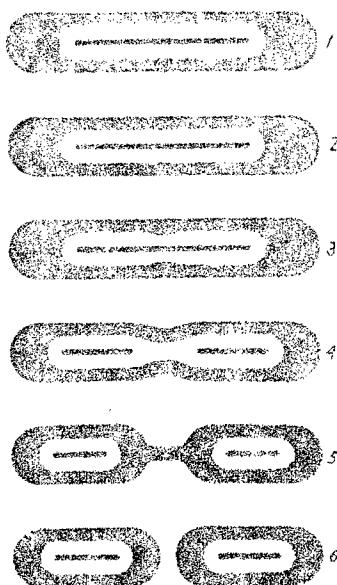


Fig. 5. Schematic of division of a bacterial nucleus.

1 -- cell at rest; 2 -- division of the nucleus begins; 3 -- division of the nucleus continues; 4 -- division of the chromatin rod and beginning of membrane narrowing; 5 -- end of division of the nucleus; 6 -- cell division completed.

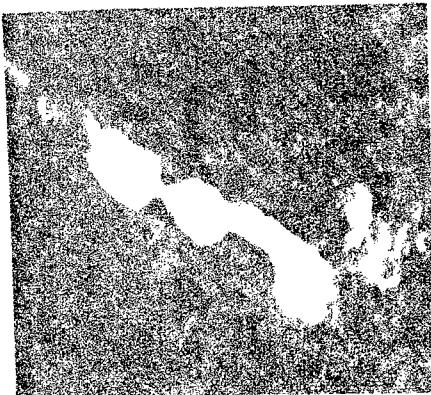


Fig. 6. Large cytoplasmic granules and "flaps" and cells after destruction by sound waves.

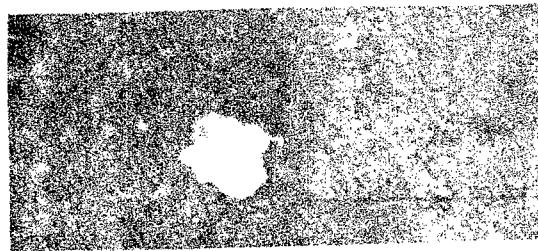


Fig. 7. A large, free cytoplasmic granule; *Bacterium suipestifer*.

view of their microchemical properties, could be regarded as nuclear. In bacterial preparations with the Romanovskiy-Gimza stain, these formations are distinguished by their intense red-violet color against a background of light pink protoplasm. The majority have a structure which stretches parallel to the length of the cell. Some are characterized by an ellipsoid or spheroid shape, and these appear in two or three formations. In isolated cells there are encountered at the poles intensely stained violet granules of the "polar cap" type which are analogous electrono-optically to the solid granules detected with an electron microscope during the study of intestinal and other micro-organisms. In bacterial preparations with the Fel'gen stain, following fixation with Carnois liquid an analogous picture was seen. In the field of such preparations there were seen poorly stained, lilac-blue bacilli with nuclear elements in the form of red-violet enclosures. These enclosures resemble globules or short rods situated longitudinally, transversely, and diagonally in the cells.

In the study of the bacterial sections in the electron microscope, apparent form of the nuclear structures is determined by the direction of the cut, i. e., basically longitudinal, oblique or transverse. The most complete nuclear structures are observed on bacterial sections in the longitudinal direction (see Fig. 1). Each appears as a unique vacuole in the center of the cytoplasm, in which is enclosed electrono-optically solid rods stretched along almost the entire length of the cell. On sections of many *B. coli aerogenes* cells, we observed, together with nuclear structures, polar cytoplasmic granules. Cells cut during the division stage usually have two rods enclosed in the vacuoles, one in each half of the dividing cell. On the transverse sections of the bacteria the nuclear structures are small, round formations representing rods cut transversely (see Fig. 1). On oblique sections the nuclear structures appear in the form of short round rods with edges cut at an angle.

The comparison of the external views of nuclear structures in the sections, arranged in various directions, shows that these structures are solid round rods enclosed in the zone of the substance (vacuole), which has almost zero density for the electrons. This substance, it seems to us, corresponds to the caryolymph (nuclear fluid) of higher-type cells.

The external appearance and character of distribution of nuclear structures on bacterial sections, as elicited with electron microscopy, are analogous to the type and character of structures elicited in sections by means of

staining and microchemical analysis with subsequent light microscopy. The longitudinal sections show clearly a slightly pink protoplasm, and against this background, red-violet nuclear formations in the form of twigs, and sometimes also violet "polar caps." In cells with bands in the middle, the nuclear formations appear in pairs, corresponding to the halves of the dividing cell. In the transverse bacterial sections with Romanovskiy-Gimza stain, the nuclear formations appear in the form of discreet violet enclosures.

Thus, the combined study of the sections shows that the electrono-optically solid rods are stained with specific dyes and can be considered chromatic.

In the experiments with *B. coli* B sections we attempted to follow up the finer composition of nuclear structures as well as to find further proof of their division, since the appearance of the nuclear structures in the sections of the dividing *B. coli* aerogenes cells leads to the conclusion that the nuclei undergo division.

Despite the fact that the observations and photography were carried out under high power (about 15A), we did not succeed in eliciting the presence of membranes containing nuclear formations, although the cell walls and cytoplasmic membranes in the sections at this magnification were detected clearly. Nevertheless, the borders between the fine-grained cytoplasm and the nuclear content were very clearly visible, as seen in the photographs, which enabled us to get an idea of the area occupied by the nuclear formation in relation to the total area of the bacterial cell section. Other authors (Chapman and Hillier, 1953; Maale, Birch-Anderson and Sjostrand, 1954) also were unable to elicit the membrane of the nuclear apparatus. The presence of a definite order between the nuclear structures and the cytoplasm, and the possibility of employing structural analysis at higher powers constitute the basis for further research.

A study of numerous pictures of sections showed that the size of the longitudinal chromatin rods are not uniform in various cells. The thickness of the rods varies between 120 and 720 A, while the length sometimes reaches 500 A and more. None of the pictures showed rods directly adjacent to the cytoplasmic border. Usually the ends are situated at a slight distance from this border. In the pictures they have the character of solid structures that give no evidence of being composed of smaller particles.

The division of nuclear structures is easily followed on sections of dividing bacterial cells. In the cells, where the process of division is clearly expressed, the nuclear substance is present in the form of bright zones with solid rods enclosed in each half of the dividing cells, or cells

which are divided except that a small connecting tie of the cell membrane is retained. On the other hand, in studying our serial ultrafine *B. Coli* B sections, we became convinced that the process of division of nuclear formations starts before the appearance of any signs of the division of the cell membrane or cytoplasm. Moreover, the division of nuclear formations in cells which have just been formed by division takes place before division of the initial cell is complete. Thus, the division of nuclear structures starts and ends earlier than the division of the entire bacterial cell in which they are located.

Thus, our data show that nuclear elements are present in the cytoplasm in a distinct state independently of "polar caps" of which there can be more than two. However, in contrast to M. A. Peshkov (1955) who had worked with a light microscope, we found that the nuclear structures in electron-microscopic observations always appear as longitudinal rods which correspond to the bacillary nuclear elements observed in the study of bacteria under a light microscope. The elicitation of spherical nuclear structures, described by many authors, is due to a particular arrangement of cells of the slides in the fixation of smears. For instance, in a cell fixated perpendicularly to the surface of the slide, the nuclear formations will always appear spherical, as is seen on the transverse bacterial sections. Thus the reports of various forms of nucleoids are due, apparently, to observation of various arrangements of bacteria on the surface of the preparation, and do not reflect the true form of the nuclear structures.

Hence, it is understandable that contradictions appeared in the literature between the results of photo-visual and electron-microscopic observations.

Nuclei were always elicited in the study of ultrafine bacterial sections. We stress this fact, since it is basic in confirming the general concept of the existence of a bacterial nucleus. In our opinion, the existence of distinct nuclear bacterial structures must be considered definitely proved in the light of modern data. The bacterial nuclear structure can be regarded as comprising unique vacuoles which, being situated in the central part of the cells, are filled with material easily penetrated by electrons and containing solid chromatin rods longitudinally extended (A. P. Pekhov and G. I. Fedorova, 1959).

The above-cited data on the division of the nuclear bacterial structure clearly indicate the leading role of this process in the division of the entire bacterial cell, similar to that of the process which takes place in the proliferation of cells of higher forms. In analyzing the morphological

pictures of the dividing cells on serial ultrafine sections, we arrive at the conclusion that the division of nuclear structures, the first step in bacterial cell division, corresponds in its general outlines to the scheme shown in Fig. 5, which can be defined as amytosis. True, this fact contradicts De Lamater (1957) who, on the basis of photo-visual and microchemical observations, thinks that the bacterial nucleus divides caryokinetically.

In comparing the data on bacterial nuclear structures with the data on yeast, seaweeds and animal and planter cells, we can find a number of common features. Nevertheless, to draw a complete analogy seems premature, although some authors (Piekarski and Giesbrecht, 1956) pose the question, based on the similarity of manifestations, of the possibility of substituting the "nucleoid" concept, generally employed in the literature in the designation of nuclear bacterial structures, for "nucleus," for the purposes of complete identification of the structure. Such a view can not be factually substantiated for a number of reasons. In the first place, up to the present no membrane has been elicited in bacterial nuclear structures. This circumstance represents a very important differentiating characteristic in a simplified analysis of the organization of bacterial nuclear structures. Also, bacteria lack a chromosome arrangement which would be similar to the one observed in cells of higher forms. Up to the present time no experimental work has proved the presence of an arrangement of this type.

And, finally, though some isolated chromatin rods of the nuclear apparatus of the bacteria are apparently of the nature of chromosomes, they nevertheless differ structually from chromosomes of cells of higher forms. In the division of bacterial nuclear structures, amytosis is the mechanism involved.

Thus the bacterial nuclear apparatus, though having much in common with the nuclei of cells of higher forms, is characterized by its own peculiarities which, however, do not prevent it from being regarded as a true nucleus. The presence of an organized nucleus in bacteria, and the possibility of quantitative determination of the relation between it and the cytoplasm, place the caryology of bacteria on a firm foundation and create the premises for further study of some very important problems such as, for example, polyploidia and the polyploid mutants of bacteria.

(b) Large cytoplasmic granules, equivalent to mitochondria. As noted above, a problem has been posed in recent years in the literature in regard to the large cytoplasmic bacterial granules which, because they are located most frequently at the poles, are called polar granules or

"polar rods." The difference between these and nuclear structures has been mentioned already. In the literature they also called volute, metaphosphate granules, or Babesh-Ernest corpuscles, depending on the type of the microorganism.

In observing intact bacteria under an electron microscope, these granules appear as solid, well-delineated spheroid or ellipsoid formations situated usually at the poles against a background of less solid cytoplasm. According to the data of Mudd (1953), the size of these granules is not the same in all microorganisms. In various mycobacteria the diameter of the granules is 0.02-0.05 microns, in corynebacteria 0.7-0.0 sic microns and over, and in *B. coli* 0.2-0.4 microns. The number of granules in a cell also varies. Usually, there are cytoplasmic granules situated on each pole, but sometimes there are more, in which case they are situated along the longitudinal bacterial axis.

The outstanding characteristic property of large cytoplasmic granules is their resistance to physical and biological influences.

In carrying out an analysis of the effect of high-frequency sound on *suipestifer* cells (1956), we found that these granules are the most resistant component of the bacteria. While microbial cells still preserved their bacillary form, the protoplasm became very transparent, but the granules under these circumstances showed no disintegration or change in density (Fig. 6). The granules remained intact even after complete disintegration of the bacteria subjected to sound vibrations and, having become liberated from the cells, appeared as free formations similar in density and size to the granules of the intact cells (Fig. 7). Recently similar results have been obtained in an electron-microscopic analysis of sound-treated preparations of another type of bacteria (Carr, 1958).

Large cytoplasmic granules remain morphologically intact also following phagolysis of bacteria, as was demonstrated by some researchers in the lysis of *B. coli* B with the T2 phage (Hartman, Mudd, Hillier, Beutner, 1953) and by us on the system of the phagobacterium *B. coli aerogenes* (1957).

Certain data on the chemistry and cytochemistry of these granules are of importance for their functional characterization. Under intensive bombardment by the stream of electrons in the column of the microscope the granules are "sublimated" and leave behind destroyed membranes (Mudd, Winterscheid, De Lamater, and Henderson, 1951), which indicates the presence of metaphosphates in the granules, since a similar "sublimation" is obtained, as is known, with electron bombardment of mixtures of sodium metaphosphate and ribonucleate of calcium. The presence of phospholipids was also detected (Davis, Winterscheid, Hartman, Mudd, 1953).

Numerous cytochemical data show that the large cytoplasmic granules contain coordinated systems of oxidation-reduction enzymes. In the staining of microbial cells of various types with tetrazole salts, the latter are reduced to their chromatic formazans at the expense of granules. In our experiments the specific staining of granules in cells subjected to the effects of sound and phages was obtained with the use of Janus green.

Thus, large cytoplasmic granules are characterized by a definite morphological autonomy, high resistance, and specific biochemical features. This enables us to consider as perfectly legitimate the idea that large cytoplasmic granules have special significance as cellular components of the bacteria. Even before these data were obtained, the opinion was advanced (Graffi and Maas, 1940; King and Beams, 1942) that they are equivalent to mitochondria.

The present factual data, part of which are described here, renders valid the contention that these granules are truly the equivalents of mitochondria in animal and plantar cells (Mudd, 1953).

We have analyzed here, though far from completely, the basic results of the research work in the study of the submicroscopic organization of bacteria. The basic conclusion arrived at from these studies is the fact that there are substantial similarities between the bacterial cell and the cells of higher organisms. The importance of this deduction is unquestionable.

The present level of knowledge of submicroscopic bacterial organization, as well as the possibilities of electron microscopy as a very precise method of ultrastructural analysis of matter, make it possible to speak of creating now a new branch of microbiology, the submicroscopic cytology of bacteria, with appropriate objects and methods of investigation.

A further, more thorough ultrastructural characterization of cellular bacterial components, combined with a more detailed study of their biochemical and genetic characteristics, will constitute most important additions to the knowledge of the nature of bacterial properties and the manifestations they produce in bacterial life processes.

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